High-sensitivity SARS-CoV-2 S1 IgG ELISA Kit (CAT NO: 41A232)

For quantitative determination of IgG against SARS-CoV-2 Spike S1 protein in serum, plasma or other specimen

This package insert must be read in its entirety before using this product

Version: 20200520

BACKGROUND

SARS-CoV-2 is an enveloped virus with a positive-sense RNA genome and a nucleocapsid of helical symmetry. The SARS-CoV-2 entry into host cells is mediated by the transmembrane spike
(S) glycoprotein, which is the main target of neutralizing antibodies upon infection and the focus of the therapeutic and vaccine design. S comprises two functional subunits responsible for binding to the host cell receptor (S1 subunit) and fusion of the viral and cellular membranes (S2 subunit). The distal S1 subunit comprises the receptor-binding domain(s) and contributes to stabilization of the prefusion state of the membrane-anchored S2 subunit that contains the fusion machinery.

INTENDED USE

SARS-CoV-2 S1 protein IgG ELISA Kit is a highly sensitive and specific immunoassay developed by TorontoBioscience for the precision detection and quantitative measurement of IgG class antibodies against the spike S1 protein of SARS-CoV-2 virus in human blood samples in vitro.

This product is intended for used by professional persons only.

This product is intended for research use only.

ASSAY PRINCIPLE

96-well plates are coated with SARS-CoV-2 S1 protein that captures IgG antibodies against SARS- CoV-2 S1 protein in the sample. After washing away unbound materials, captured IgG against SARS-CoV-2

S1 protein is detected by anti-human IgG polyclonal antibodies conjugated with horse radish peroxidase (HRP). After washing step, the chromogenic substrate 3,3',5,5'- tetramethylbenzidine (TMB) is added. Color reaction is stopped by 2M H2SO4. The amount of anti- SARS-CoV-2 S1 antibodies captured inside the wells is proportional to the color density generated in the coupled oxidation-reduction reaction. The unknown sample concentration can be interpolated from a standard curve generated by Human anti-S1 monoclonal antibody (mAb).

REAGENTS SUPPLIED

Each kit is sufficient for 96 tests and contains the following components:

1. One aluminum pouch with a Microwell plate (12 strips of 8 wells each) coated with SARS- CoV-2 S1 protein, sealed. The microwell strips can be used separately.

2. 10×Wash buffer-40 ml.

3. 5×Assay buffer-20 ml.

4. 100×Detection antibody solution: HRP-conjugated anti-human IgG, 0.12 ml.

5. 10 × Standard: Humanized anti-S1 mA- 50 µL.

6. Substrate solution, 12 ml, ready for use.

7. Stop solution, 12 ml, ready for use.

OTHER MATERIALS REQUIRED, BUT NOT PROVIDED

1. Pipettes and pipette tips.
2. Beakers, flasks, cylinders necessary for preparation of reagents.
3. Buffer and reagent reservoirs.
4. Paper towels or absorbent paper.
5. Plate reader capable of reading absorbency at 450 nm.
6. Distilled water or deionized water.
7. Statistical calculator with program to perform regression analysis.

STORAGE

- The kit should be stored at 2-8°C, and all reagents should be equilibrated to room temperature before use. Immediately after use remaining reagents should be returned to cold storage (2- 8°C).
- Expiry of the kit and reagents is stand on labels.
- Once opened, the strips may be stored at 2-8°C for up to one month.

SAMPLE COLLECTION AND STORAGE INSTRUCTIONS

Handle serum or plasma sample in accordance with National Committee for Clinical Laboratory Standards guidelines for preventing transmission of blood-borne infection.

- Do not use grossly hemolyzed or lipemic samples.
- Human Serum: Use a blood separator tube and allow sample to clot for 30 minutes, then centrifuge for 10 minutes at 1000g. **When the human serum is tested, it should be diluted 100-fold at least.**
- Human plasma: Treat blood with anticoagulant such as citrate, EDTA or heparin. Centrifuge for 10 minutes at 1000g within 30 minutes for plasma collection. **When the human plasma is tested, it should be diluted 100-fold at least.**
- Samples cannot be tested immediately should be aliquoted and must be stored frozen below - 20°C. Avoid repeated freeze-thaw cycle.
- Perform preliminary experiment to determine the optimum detection sample dilution.

PRECAUTIONS FOR USE

- All chemicals should be considered as potentially hazardous. Avoid contact with skin and eyes. In the case of contact with skin or eyes wash with water.

- Do not use kit reagents beyond expiration date.
- Do not expose kit reagents to strong light.
- Do not pipet by mouth.
- Do not eat or smoke in area where kit reagents or samples are handled.
- Use only sufficient volume of specimen as indicated in the procedure steps. Failure to do so, may cause low sensitivity of the assay.
- Avoid contact of substrate solution with oxidizing agents and metal.
- Use disposable pipette tips and/or pipettes.
- Use clean, dedicated reagent trays for dispensing the conjugate and substrate reagent.
- Do not touch the exterior bottom of the wells; fingerprints or scratches may interfere with the reading. When reading the results, ensure that the plate bottom is dry and there are no air bubbles inside the wells.
- The enzymatic activity of the HRP-conjugate might be affected from dust and reactive chemical and substances like sodium hypochlorite, acids, alkalis etc. Do not perform the assay in the presence of these substances.
- Substrate solution must be at room temperature prior to use.

PREPARATION OF REAGENTS

Bring all reagents and materials to room temperature before use

1. 1×Wash buffer.

Prepare 1×Wash buffer by mixing the 10×Wash buffer (40 ml) with 360 ml of distilled water or deionized water. **【CAUTION!!!】If precipitates are observed in the 5x assay buffer bottle, warm the bottle in a 37°C water bath until the precipitates disappear. Incomplete dissolution will lead to high background.** The 1×Wash buffer may be stored at 2-8°C for up to one month.

2. 1×Assay buffer.

Prepare 1x assay buffer by mixing the 5x assay buffer (20 ml) with 80 ml of distilled water or deionized water. **【CAUTION!!!】If precipitates are observed in the 10xWash buffer bottle, warm the bottle in a 37°C water batch until the precipitates disappear. Incomplete dissolution will lead to high background.** The 1x assay buffer may be stored at 2- 8°C for up to one month.

3. 1×Detection antibody solution.

Spin down the 100×Detection antibody solution briefly and dilute the desired amount of the antibody 1:100 with 1×Assay buffer, 100 µL of 1×Detection antibody solution is required per well. Prepare only as much 1×Detection antibody solution as needed. Return the 100×Detection antibody solution to 2-8°C immediately after the necessary volume is pipetted.

PREPARATION OF STANDARD AND SAMPLES

Standard preparation:

Centrifuge the standard tube briefly before opening the cap. Add 450 µL 1 × assay buffer into Anti-S1 mAb 10× standard (50 µL) to generate the first standard (10 ng/ml). Prepare serially diluted standards using 1 × assay buffer as follow:

	Standard Volume	Volume of 1 × assay buffer	Concentration
1	10 ng/ml	-	10 ng/ml
2	250 µL of 10 ng/ml	250 µL	5 ng/ml
3	250 µL of 5 ng/ml	250 µL	2.5 ng/ml
4	250 µL of 2.5 ng/ml	250 µL	1.25 ng/ml
5	250 µL of 1.25 ng/ml	250 µL	0.625 ng/ml

1x Assay buffer serves as the blank (0 ng/ml).

Note: The reconstituted standard stock should be aliquoted and stored at 2-8 ℃ for up to one month.

Sample preparation:

Serum or plasma sample is generally required a **100-fold dilution** in the 1X Assay buffer. A suggested dilution step is to add 2 µL of sample to 198 µL of 1X Assay buffer. Dilution factor can be adjusted based on the titre of the antibodies in the samples.

ASSAY PROCEDURE

It is recommended that all standards and samples be assayed in duplicate.

1. Add 100 µl of standards or samples per well, and incubate at room temperature for 1 hour, with shaking at 600 rpm. (Note: incubation without shaking proportionally decreases the signal by approximately 30% If no shaker available, prolong the incubation time to 2 hours).

2. Discard the content and tap the plate on a clean paper towel to remove residual solution in each well. Add 300 µl of 1×Wash buffer to each well and incubate for 1 minute. Discard the 1×Wash buffer and tap the plate on a clean paper towel to remove residual wash buffer. Repeat the wash step for a total of 4 times.

3. Add 100 µl of 1×Detection antibody solution to each well, incubate at room temperature for 1 hour.

4. Wash each well 4 times as described in step 2.

5. Add 100 µl of Substrate solution to each well, incubate at room temperature for 15 minutes. Protect from light.

6. Add 100 µl of Stop solution to each well, gently tap the plate frame for a few seconds to ensure thorough mixing.

7. Determine the optical density of each well at 450 nm immediately.

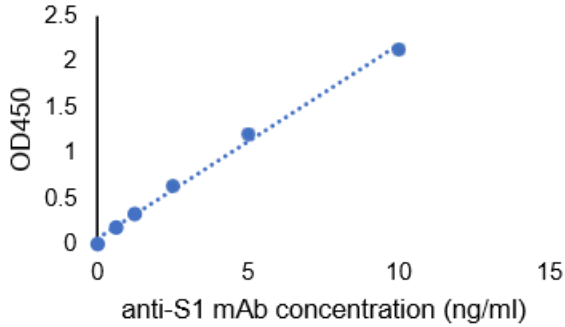
CACULATION

1. Subtract the absorbance of the blank from that of standards and samples.

2. Generate a standard curve by plotting the absorbance obtained (y-axis) against Human anti-S1 mAb (x-axis). The best fit line can be generated with any curve-fitting software by regression analysis. Any curve of 4-parameter or log-log curve fitting can be used for calculation.

3. Determine human anti-S1 IgG concentration of samples from standard curve and multiply the value by the dilution factor.

TYPICAL STANDARD CURVE



PRECISION

Intra-assay: Three different known levels of positive control were spiked into sample buffer as test samples. All samples were tested on the same plate to evaluate intra-assay precision of the kit. The Intra-assay precision of this kit is less than 5%.

Inter-assay: Three different known levels of control were spiked into sample buffer as test samples. All samples were tested in 3 separate assays to evaluate intra-assay precision of the kit. Inter-assay precision of this kit is less than 7%.

SENSITIVITY AND SPECIFICITY

We have tested this assay in blood samples of 60 well-characterized COVID-19 patients, and 58 of 60 patients were anti-SARS-CoV-2 S1 IgG positive. 20 blood samples from healthy individuals were tested, and none of them were positive. **The sensitivity of this assay is ⩾ 96%, and the specificity of this assay is ⩾95%.**

REPRESENTATIVE DATA FROM COVID-19 PATIENTS (n=20) AND HEALTHY INDIVIDUALS

COVID-19 patient				Healthy individuals			
No	OD450	No	OD450	No	OD450	No	OD450
1	0.626	11	0.595	1	0.078	11	0.148
2	0.363	12	2.139	2	0.148	12	0.124
3	0.369	13	1.948	3	0.088	13	0.069
4	3.967	14	2.004	4	0.094	14	0.083
5	2.731	15	0.462	5	0.104	15	0.126
6	1.948	16	0.234	6	0.131	16	0.093
7	0.551	17	1.079	7	0.094	17	0.088
8	1.309	18	3.721	8	0.074	18	0.068
9	0.494	19	0.903	9	0.113	19	0.121
10	1.776	20	3.130	10	0.13	20	0.186

SUMMARY OF ASSAY PROCEDURE

Add 100 µl of standard/sample to each well.

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Incubate at room temperature for 1 hour with shaking at 600 rpm. (If shaker is not available, prolong the incubation time to 2 hours.)

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Aspirate and wash each well three times.

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Add 100 µl of 1×Detection antibody solution to each well.

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Incubate at room temperature for 1 hour.

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Aspirate and wash each well four times.

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Add 100 µl of Substrate solution to each well.

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Incubate at room temperature for 15 minutes.

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Add 100 µl of Stop solution to each well.

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Measure absorbance of each well at 450 nm.

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Calculation and Interpretation

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